2177-Plat
Single Molecule RNA Base Identification with a Biological Nanopore
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The sequencing of individual DNA and RNA strands with nanopores is under investigation as a rapid, low-cost platform. In this approach, bases are identified in order as the strand is transported through a pore under an electrical potential. Although the preparation of solid-state nanopores is improving, biological nanopores, such as α-hemolysin (αHL), are advantageous because they can be precisely manipulated by genetic modification. Previously, it has been shown that the αHL nanopore contains three recognition sites, capable of discriminating between single DNA bases when oligonucleotides are immobilized within the nanopore. Here we extend these investigations into discrimination of nucleobases in RNA. By immobilizing RNA homopolymers within the αHL pore using a terminal biotin-streptavidin complex, we achieve sharply defined current distributions that enable clear discrimination of the nucleobases. This is further extended by investigating individual RNA bases in a DNA polyC background, which gives comparable results. Additionally, with a view to ''exo sequencing'', an engineered protein nanopore is successfully used to detect and discriminate all four ribonucleoside monophosphates (rNMPs) by using, am7-β-cyclodextrin as both a transient and covalent molecular adapter.

2178-Plat
Nanopore Analysis of Individual RNA/Antibiotic Complexes
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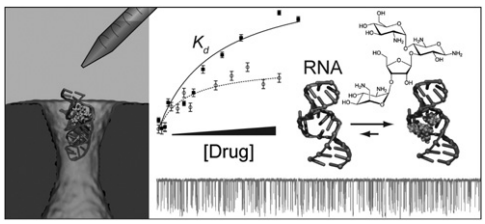
Nanopores in thin solid-state membranes are used to rapidly analyze individual RNA/drug complexes. The interactions of a truncated A-site RNA model of the prokaryotic ribosome with aminoglycoside antibiotics are characterized by passing individual molecules through a 3-3.5 nm diameter pore fabricated in a 8-10 nm thick silicon nitride membrane. Complexes of the A-site RNA with aminoglycosides can be distinguished from unbound A-site based on the ion current signatures produced as they pass through the nanopores. Counting the fraction of free and drug-bound molecules affords label-free drug-RNA binding isotherms consistent with literature reports and with data generated using independent fluorescence-based assays. Our measurements are supported by molecular dynamics simulations, illustrating a relationship between the ionic current and complexation of paramomycin, a prototypical aminoglycoside antibiotic, with the A-site RNA construct.



2179-Plat
Developing Nanopores with Fluid Walls for Improved, Single-Molecule Biosensors
Erik C. Yusko[1], Alex M. Petti[1], Panchika Prangkio[1], Ryan C. Rollings[2], Jiali Li[2], Jerry Yang[3], Michael Mayer[1].
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Synthetic and biological nanopores can be used for fundamental and applied studies of individual biomolecules in high throughput. By measuring resistive current pulses during the translocation of single molecules through an electrolyte-filled nanopore, this technique can characterize the size, conformation, assembly and activity of hundreds of unlabeled molecules per second. The performance of nanopores fabricated in synthetic materials, however, does not currently match that of biologic ion channels. Major challenges include: (i) achieving precise control of nanopore diameters, (ii) translocation times of molecules that are too fast to be resolved by the best electronics, (iii) a lack of control over the surface chemistry inside synthetic pores, which leads to non-specific adsorption, pore clogging, and unpredictable translocation times, (iv) low frequencies of translocation events at low analyte concentrations and (v) poor specificity of the nanopores for analytes. Inspired by the olfactory sensilla of insect antennae, we demonstrated that coating nanopores with a fluid lipid bilayer addresses all of these challenges while enabling new nanopore-based assays. Coating nanopores with different lipids allows fine control of the surface chemistry and diameter of nanopores. Incorporation of mobile ligands in the lipid bilayer imparts specificity to the nanpore for targeting proteins and enables, for the first time, precise and predictable control of translocation times for targeted proteins based on their net electric charge. Here, we detail how this modification can also be used to determine the affinity of a protein-ligand interaction as well as monitor the kinetics of binding. In addition, the biomimetic lipid surface reduced non-specific adsorption, thereby allowing characterization of Alzheimer's Disease-related amyloid peptides, for which a reliable, biophysical method is lacking.

2180-Plat
Translocation of DNA-Protein Complexes through Solid-State Nanopores
Cees Dekker.
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I will report a variety of novel results connected to translocation of DNA-protein complexes through solid-state nanopores. Specifically I will address:
1. Detection of nucleosomes on DNA. We can distinguish histone monomers, dimers and tetramers as well as single or multiple nucleosomes on DNA. This lays the basis for nanopore studies of nucleosome structure and remodeling.
2. Detection of local protein structures along DNA. I will report our recent progress in detecting single proteins along a DNA molecule as it passes the nanopore, which involves slower translocation, higher bandwidth, and improved signal-to-noise.
3. The use of through atomically thin graphene nanopores for the translocation of double-strand as well as single-strand DNA. To prevent sticking we developed a reliable protocol for a self-assembled monolayer on graphene which I will report at the meeting.

2181-Plat
Stochastic Sensing of Proteins with Receptor-Modified Solid-State Nanopores
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Nanopores bear great potential as sensors for single molecules, which are detected as resistive pulses in a simple electrical measurement. The investigation of proteins requires made-to-measure pores with customized dimensions and surface properties, a need that is best met by engineered nanopores in solid-state membranes. A prerequisite for the versatile utilization of artificial nanopores for molecular interaction studies, however, is the establishment of generic modification strategies to functionalize the pores with a receptor group featuring desired chemical properties. By this means, the pore shall adopt (bio) chemical selectivity and become affine to proteins of interest.
Here we demonstrate the stochastic sensing of proteins with metallized silicon nitride nanopores, which are chemically modified with nitrilotriacetic acid (NTA) receptors embedded in an otherwise protein-repellant self-assembled monolayer. The reversible binding and unbinding of individual antibodies and His-tagged proteins and the quantitative analysis of interaction parameters is reported. Importantly, single molecule sandwich assays can be conducted readily with an arbitrary set of proteins (provided that one of them is His-tagged), which is demonstrated exemplarily for the discrimination of IgG subclasses with a protein-modified, antibody-selective nanopore.
The introduced methods are generally applicable to create protein-selective nanopores and pave the way for the use of artificial nanopores as generic devices to study protein-protein interactions one-on-one.

2182-Plat
Protein Unfolding and Stability Measurement using a Solid-State Nanopore
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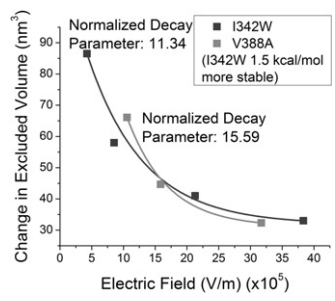
Until recently, only a few researchers have utilized solid-state nanopores for studying protein biophysics. One reason for this has been the well documented phenomenon of protein adsorption which occurs on the pore surface. In our studies, we have manipulated experimental parameters to overcome this and other issues as well as develop a new paradigm for studying protein folding and stability. Specifically, we quantitatively describe the denaturing effects of electric fields; being of fundamental importance to all nanopore experiments as well as to endogenous protein unfolding. Furthermore, due to the difficulty in separating electric-field unfolding from other unfolding mechanisms, in this study we have also developed a way to subtract out all other influences and obtain measures of chemical stability. By plotting the change in a protein's excluded



volume (8M urea minus 0M urea) as a function of voltage, we observe an exponential decay as the electric field effects override the chemical effects until finally the presence of urea does not affect the translocation properties (Protein: PDZ2 Mutants). Nanopores are a unique and powerful tool since they can accurately produce transient denaturing conditions (using electric fields) while measuring the protein's response.

Platform: Membrane Protein Function

2183-Plat

Plasticity of the Asialoglycoprotein Receptor Deciphered by Ensemble FRET and Single-Molecule Counting PALM

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The composition of many multi-subunited receptor complexes at the plasma membrane is still debated as well as the impact of changes in the receptor subunit composition. Receptor plasticity, involving changes in stoichiometry and receptor function, has been difficult to address. Here, we introduce new spectroscopic tools to dissect receptor subunit assembly of the Asialoglycoprotein Receptor. Using this highly tractable receptor model system we show that the Asialoglycoprotein Receptor can assemble into distinct oligomers at the plasma membrane and that differential subunit assembly dictates receptor specificity. With ensemble analyses of Fluorescence Resonance Energy Transfer (FRET) and analytical modeling, we quantified receptor subunit homo- and hetero-oligomerization in the living cell. Furthermore, we established single-molecule counting using Photoactivated Localization Microscopy (PALM), visualizing an asymmetric receptor subunit assembly on the single-molecule level. Our results define a probability hierarchy of oligomerization driven by different molecular motifs that entails distinct co-existent receptor subunit assemblies. The Asialoglycoprotein Receptor is involved in the clearance of thrombogenic material. The variety of potential ligands and the propensity of the subunits to form distinct oligomers may explain previous inconsistent results and underscore the importance of deciphering oligomerization in the single cell and on the single-molecule level.

2184-Plat

Signaling in the Tumor Necrosis Factor Superfamily is Driven by Formation of Receptor Networks: Unraveling the Structural and Dynamic Paradigm

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Supramolecular clustering of receptors in the tumor necrosis superfamily, including tumor necrosis factor receptor 1 (TNFR1), Fas and Death Receptor 5 (DR5) has emerged as a potentially powerful paradigm shift in the field of apoptotic signaling. The canonical view of one ligand-one receptor is giving way to a revised picture of activity-dependent receptor aggregation. However, whether clusters have a specific structural organization stabilized by specific protein-protein contacts, as opposed to non-specific aggregation, remains unknown. Owing to the difficulty of studying endogenous membrane receptors in their native states, no detailed structural information has been available to begin to address this issue. Our studies provide the first such details of macromolecular organization in ligand-induced DR5 and TNFR1 aggregates, establishing a new paradigm for transmembrane signaling that likely extends to other structurally homologous members of this important superfamily. We base our conclusions on a rigorous, innovative and multidisciplinary approach that includes cell molecular biology in human cancer and model cell lines, biophysical measurements in cellular and synthetic model systems, and state-of-the-art computational molecular modeling. Thus, we address a fundamental and open question regarding the biophysical character of supramolecular clusters in cell biology, showing that clusters should more accurately be described as highly organized networks.

2185-Plat

Entropic Tension in Crowded Membranes

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Unlike their model membrane counterparts, biological membranes are richly decorated with a heterogeneous assembly of membrane proteins. These proteins are so tightly packed that their excluded area interactions can alter the free energy landscape controlling the conformational transitions suffered by such proteins. For membrane channels, this effect can completely change the critical driving force (such as membrane tension) needed to induce the transition from the closed to the open state, and therefore influence protein function in vivo. Despite their obvious importance, crowding phenomena in membranes are much less well studied than in the cytoplasm.

Using statistical mechanics results for hard disk liquids, we show that crowding induces an entropic tension in the membrane, which influences transitions that alter the projected area and circumference of a membrane protein. As a specific case study in this effect, we consider the impact of crowding on the gating properties of bacterial mechanosensitive membrane channels, which are thought to confer osmoprotection when these cells are subjected to osmotic shock. We find that crowding can alter the gating energies by more than ≈ 2 kB T in physiological conditions, a substantial fraction of the total gating energies in some cases.

Given the ubiquity of membrane crowding, the nonspecific nature of excluded volume interactions, and the fact that the function of many membrane proteins involve significant conformational changes, this specific case study highlights a general aspect in the function of membrane proteins.

2186-Plat

Lipid-Coupled Docking of Transmembrane Substrate by the GlpG Rhomboid Protease from Escherichia Coli

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Intramembrane proteases cleave transmembrane (TM) substrates within the plane of the lipid membrane. The GlpG rhomboid protease appears to dock its TM substrates by opening laterally towards the membrane a gate formed by helix 5. A crystal structure thought to represent GlpG in a closed state indicates a lipid headgroup bound to the catalytic site. Since the lipid must unbind for the substrate to dock, the questions arise as to how fast does lipid unbinding occur, and whether there is coupling between the incoming TM substrate and the lipid bound to the active site. To address these questions we performed systematic molecular dynamics (MD) simulations of GlpG in the absence of a TM substrate, and with TM substrate at various locations relative to GlpG. We find that, indeed, the presence of the substrate causes the lipid:active site interactions to weaken rapidly, in several tens of nanoseconds. That is, the incoming substrate prepares the enzyme for docking by inducing the displacement of the active-site lipid. The structure and dynamics of the substrate docking region of GlpG depend not only on whether or not a lipid molecule is bound to the active site, but also on the presence of the TM substrate. Our results on the lipid-coupled docking of the substrate to GlpG reveal an unsuspected complexity of the lipid interactions of intramembrane proteases.

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2187-Plat

Simulation Studies of Transport of Hydrophobic Compounds through Outer Membrane Proteins of Gram Negative Bacteria

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The outer membrane of Gram negative bacteria provides an effective barrier to diffusion of both hydrophilic and hydrophobic solutes. A number of recent X-ray studies have revealed the structures of outer membrane proteins, members of the FadL family, which are responsible for transport of hydrophobic solutes. These include: FadL from Escherichia coli (PDB id 1T16), TodX from Pseudomonas putida (PDB id 3BS0) and TbuX from Ralstonia pickettii (PDB id 3BRY). These proteins family has three significant structural features: 1) an N-terminal domain which blocks the pore otherwise formed by the transmembrane β-barrel; 2) a lateral opening formed by a kink in the β-barrel; and 3) a flexible L3 loop which seems to act as entry access point into the β-barrel. Based on this, transport models have been suggested in which lateral opening provides a pathway for exit of hydrophobic solutes from the intertior of the β-barrel into the surrounding lipopolysaccharide (LPS) membrane.

To examine the barrier posed by LPS for transport of hydrophobic compounds we performed molecular dynamics potential of mean force (PMF) calculations